Oral Normal flora

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Normal flora of the human body

In a healthy human body, the internal tissues, e.g. blood, brain, muscle, etc., are normally free of microorganisms. However, the surface tissues are constantly in contact with environmental organisms and be colonized by various microbial species. The mixture of organisms regularly found at any anatomical site is referred to as the **normal flora**.

Normal flora is an aggregate of microorganisms that placed on the surface tissues which are constantly in contact with environmental atmosphere, i.e., skin and mucous membranes.

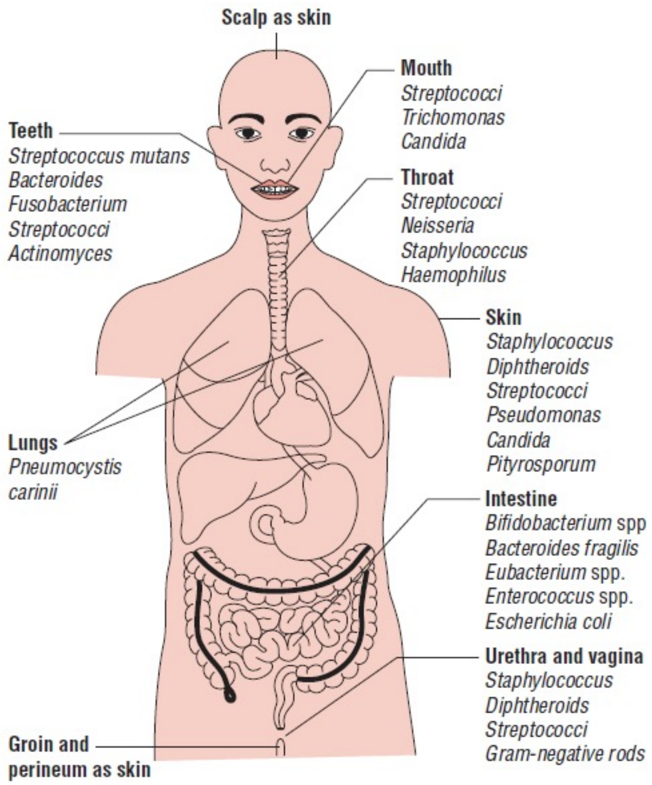
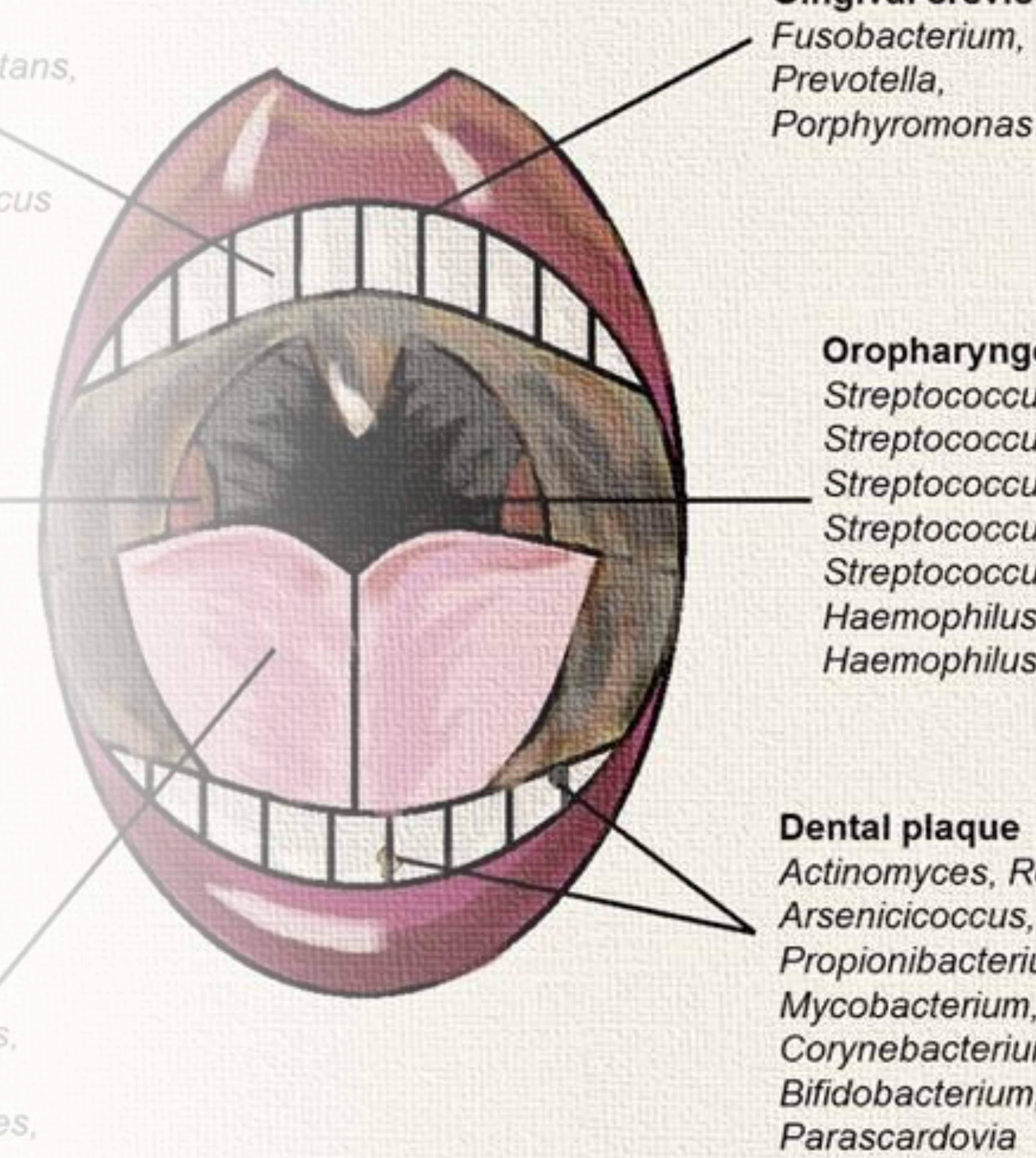


FIG. 75-1. Sites of microbial flora.

- **Oral Microbiota:**
- The Normal bacterial flora that are placed in the oral cavity is called as Oral Microbiota or Oral Microflora. Both host and bacteria are thought to derive benefit from each other, this the associations is, for the most part, **mutualistic.**

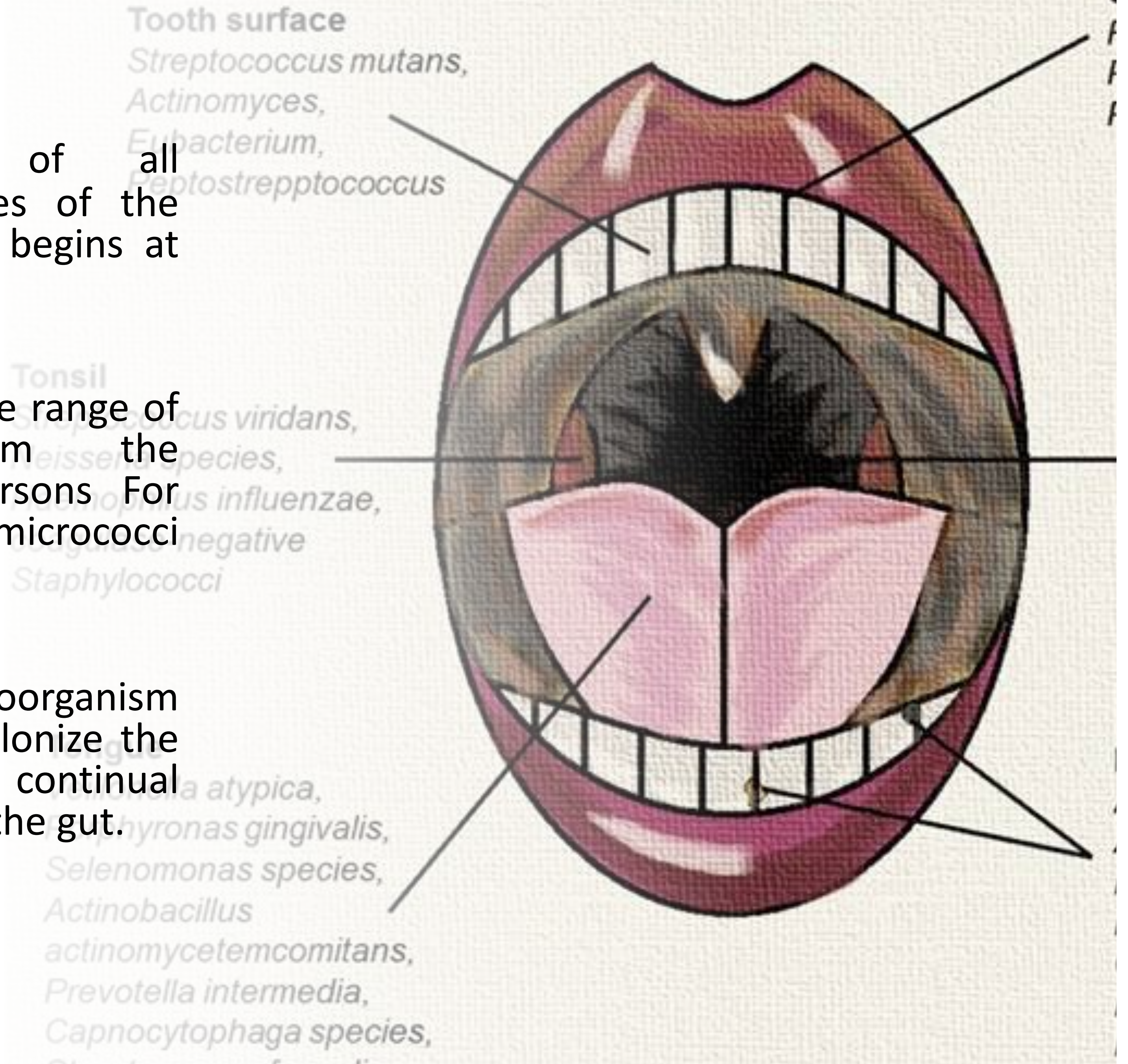
- This resident microflora does not have merely a passive relationship with its host, but contributes directly and indirectly to the normal development of the physiology, nutrition and defense systems of the organism.



- The microbial colonization of all environmentally accessible surfaces of the body (both external and internal) begins at birth.

- Such surfaces are exposed to a wide range of microorganisms derived from the environment and from other persons For example, staphylococci and micrococci predominate on the skin surface.

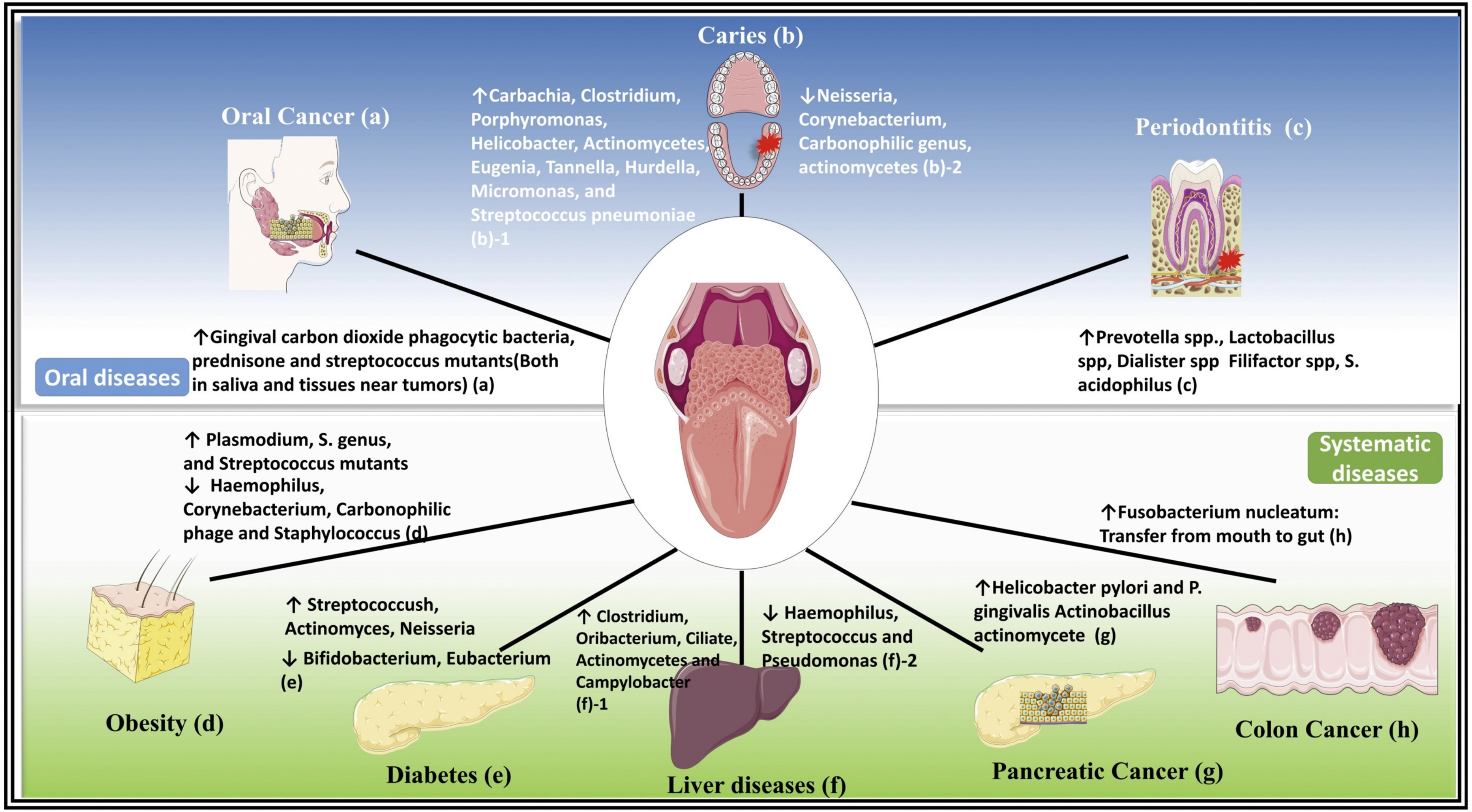
- Similarly, over 700 types of microorganism found in the mouth are able to colonize the gastrointestinal tract, despite the continual passage of these microbes through the gut.



The relationship between the normal flora and the host can be broken down in the mouth and the disease may occur. This usually due to:

- 1-Major biological changes of the mouth from exogenous sources (e.g. Antibiotic treatment) or **Endogenous changes** (Alterations in the host defenses) which affect the natural stability of the microflora.
- 2- -The presence of microorganisms at the sites not normally accessible to them as normal flora.

- As sequence of mouth-normal flora relationship disorder, Localized episodes of disease in the mouth caused by its oral microflora. The commonest clinical aspect of such imbalances are dental caries and periodontal diseases.



- **Dental caries:** is a decay of enamel or root surfaces by acid produced primarily from the metabolism of fermentable carbohydrates of the bacterial diet that are colonizing the tooth surface (dental plaque).

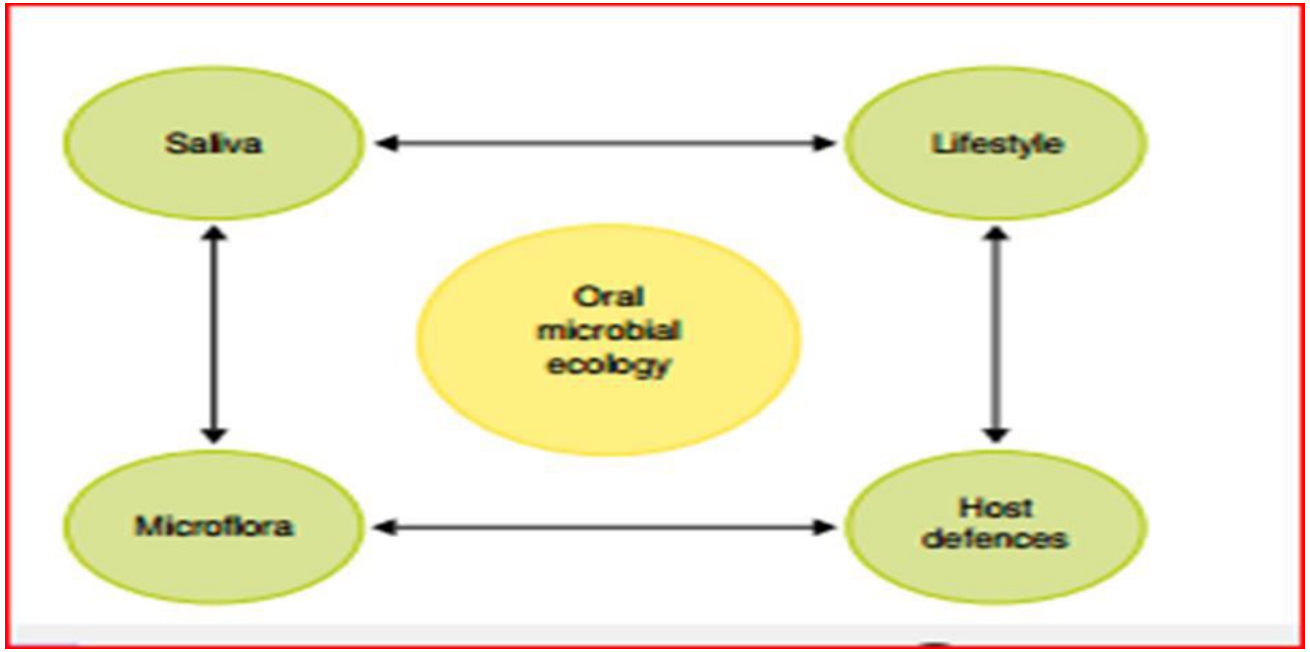
- Dental plaque is also associated with the etiology of periodontal diseases which causes an inappropriate inflammatory response to an increased microbial load (due to plaque accumulation) around the gingivae, resulting in damage to the supporting tissues of the teeth.

MICROBIAL ECOLOGY

Most diseases of the mouth have a polymicrobial (multiple species) etiology. The ability of bacteria to cause disease depends on the outcome of various interactions among the microbes themselves, and between these microorganisms and the host.

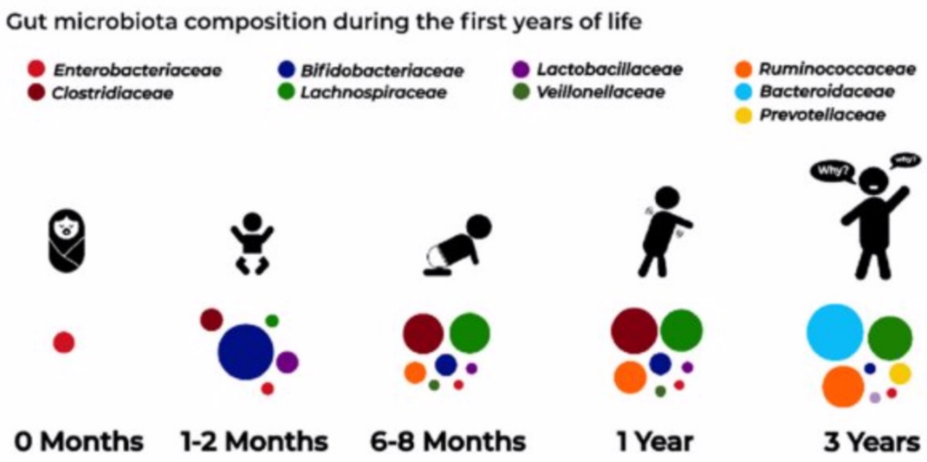
The the microbial ecology of the oral cavity is influenced by:

- 1- Flow rate and properties of saliva
- 2- The life-style of an individual (in particular, the presence of a tobacco habit, the nature of the diet, and exposure to medication)
- 3- The integrity of the host defenses.
- 4- Oral microflora



The mouth as a microbial habitat

The properties of the mouth as a microbial habitat are dynamic, and will change during the life of an individual. During the first few months of life the mouth consists only of mucosal surfaces for microbial colonization. The eruption of teeth provides a unique, hard non-shedding surface which enables much larger masses of microorganisms (dental plaque) to accumulate as biofilms. When the Gingival crevicular fluid (GCF) is produced, it can provide additional nutrients for subgingival microorganisms.



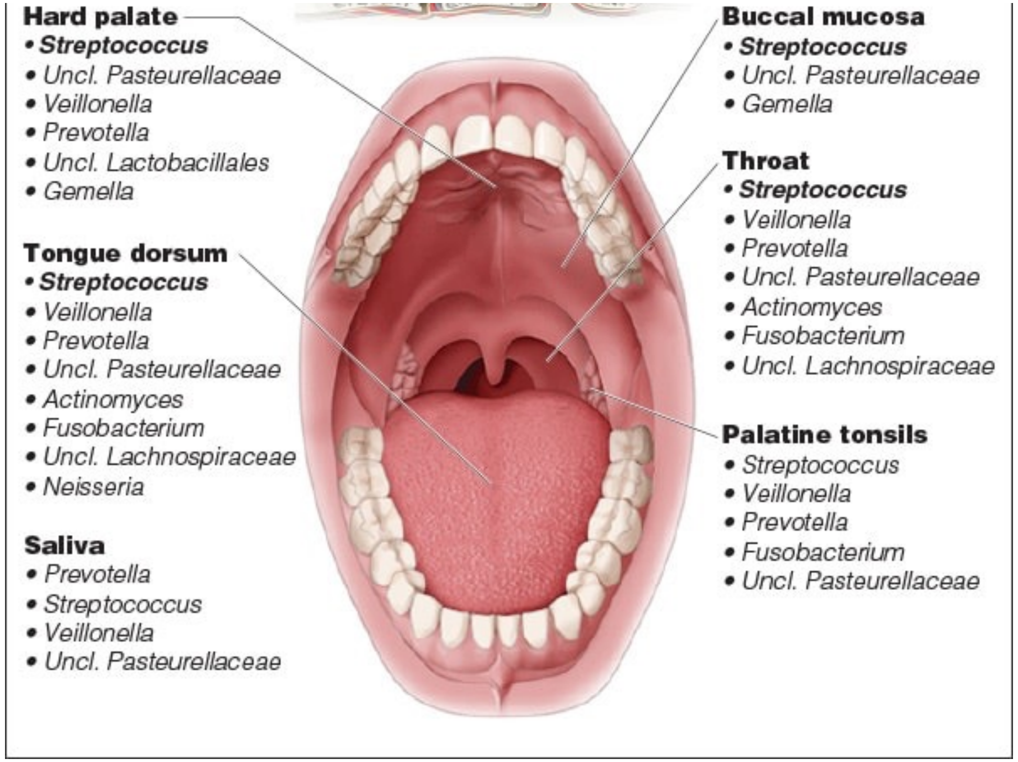
The ecology of the mouth will change over time due to the eruption or extraction of teeth, the insertion of orthodontic bands or dentures, and any dental treatment including scaling and restorations.

Transient fluctuations in the stability of the oral ecosystem may be induced by the frequency and type of food ingested, variations in saliva flow, and courses of antibiotic therapy.

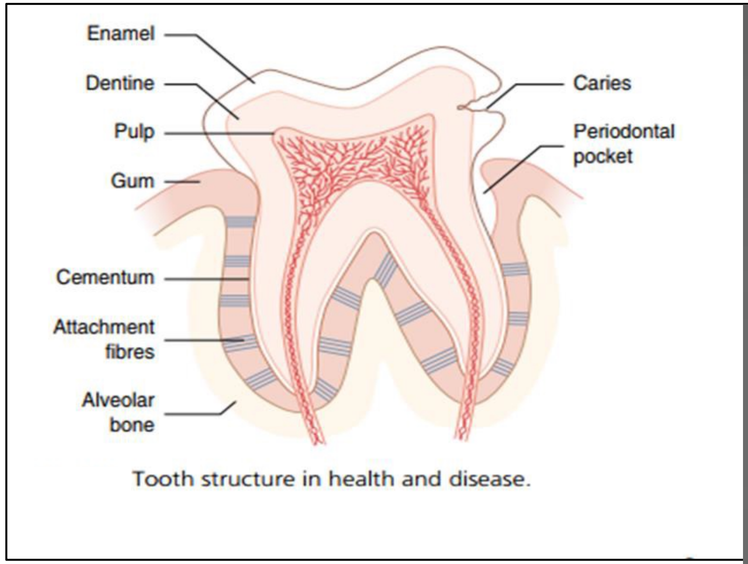
Four features that help to make the oral cavity distinct from other areas of the body are: **specialized mucosal surfaces, teeth, saliva and gingival crevicular fluid.**

- **Mucosal surfaces**

The mouth is similar to other ecosystems in the digestive tract in having mucosal surfaces for microbial colonization. The microbial load is relatively low on such surfaces due to desquamation. The papillary structure of the tongue dorsum provides refuge for many microorganisms which would otherwise be removed by mastication and the flow of saliva. Such sites on the tongue can also have a low redox potential which enable obligately anaerobic bacteria to grow and act as a reservoir for some of the Gram negative anaerobes that are implicated in the etiology of periodontal diseases and are responsible for malodor. The mouth also contains keratinized and non-keratinized stratified squamous epithelium which may influence the oral distribution of some microorganisms.



- **Teeth :**Teeth (and dentures) allow the accumulation of large masses of microorganisms and their extracellular products, termed dental plaque. In disease, there is a shift in the composition of the plaque microflora away from the species that predominate in health. With ageing, recession of the gingival tissues can expose cementum to microbial colonization and disease.



Tooth structure in health and disease.

Teeth do not provide a uniform habitat but possess several distinct surfaces. These surfaces are optimal for colonization and growth by different populations of microorganism.

1- The stagnant areas between adjacent teeth **(approximal)** and the gingival crevice afford most protection to colonizing microorganisms in the mouth . Both sites are also anaerobic and, in addition, the gingival crevice region is bathed with the nutritionally-rich gingival crevicular fluid (GCF), particularly during inflammation.

2- Smooth surfaces are more exposed to the environment and can only be colonized by a limited number of bacterial species.

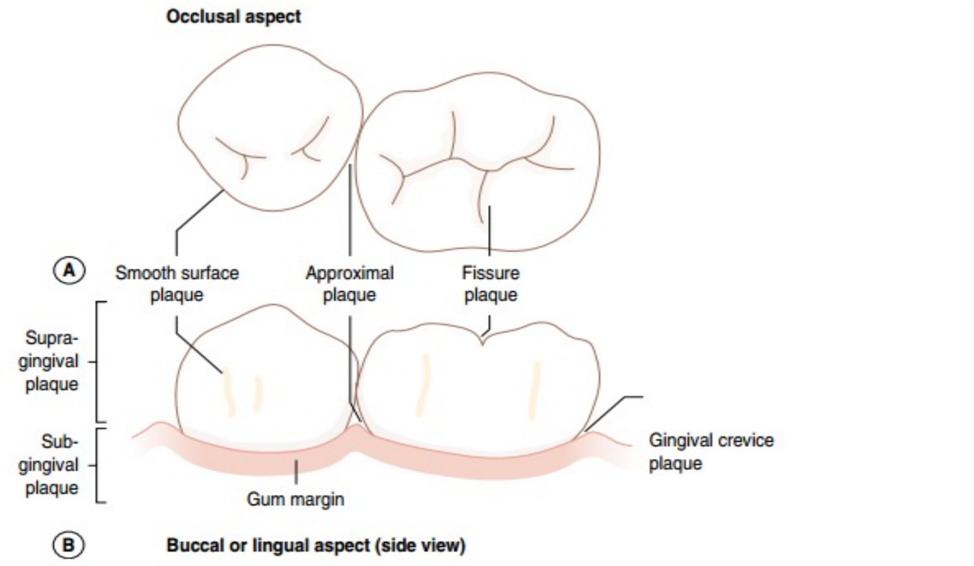


Diagram illustrating the different surfaces of a tooth, and the terminology used to describe dental plaque sampling sites.

3- Pits and fissures of the biting (occlusal) surfaces of the teeth also offer protection from oral removal forces such as saliva flow, and can contain impacted food debris. Environmental conditions on the tooth also vary in health and disease . For example, as caries progresses, the advancing front of the lesion penetrates the dentine.

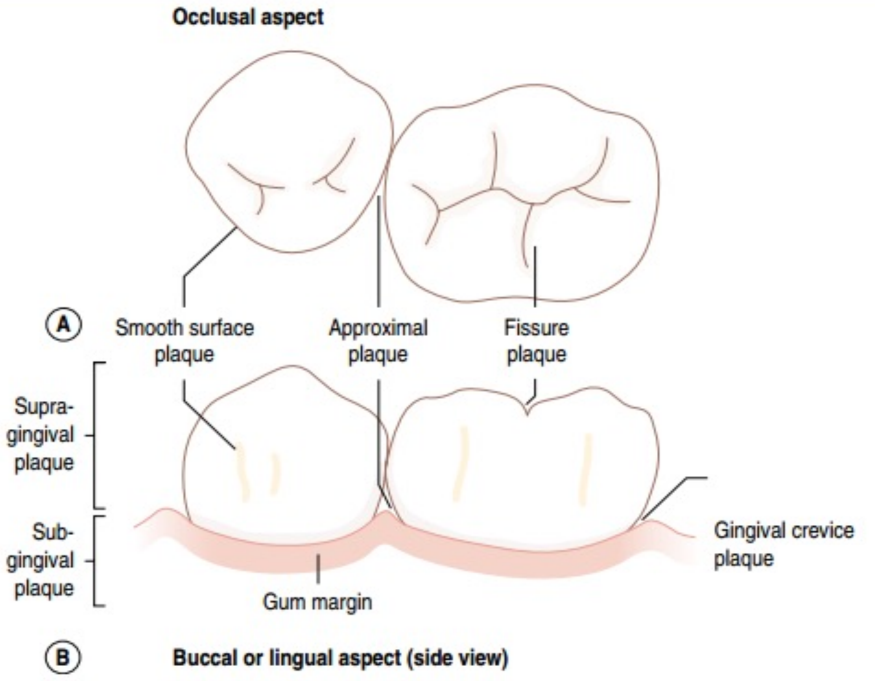


Diagram illustrating the different surfaces of a tooth, and the terminology used to describe dental plaque sampling sites.

- The nutritional sources can be changed and local conditions may become acidic and more anaerobic due to the accumulation of bacterial metabolism products. Similarly, in disease, the gingival crevice is developed into a periodontal pocket and the production of GCF is increased. These new environments will select the microbial community most adapted to the prevailing conditions. there for change in the local environment possibly resulting in a shift in the composition and metabolism of the microflora.

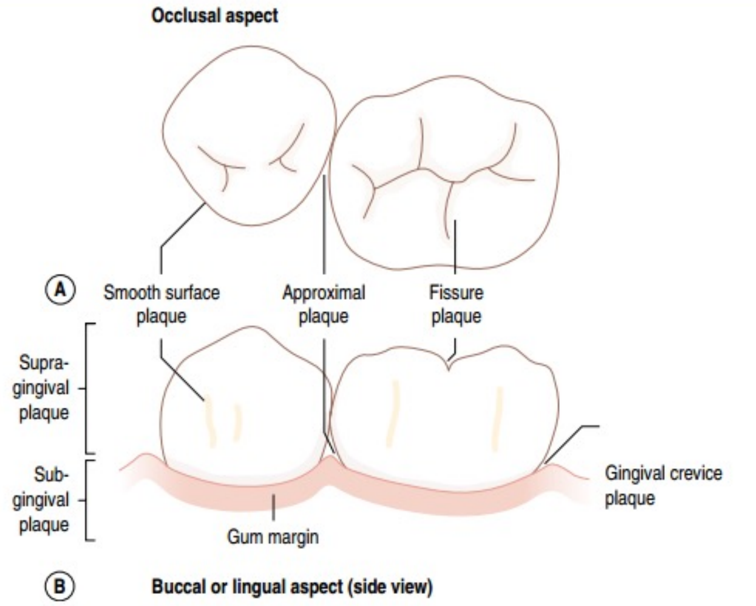


Diagram illustrating the different surfaces of a tooth, and the terminology used to describe dental plaque sampling sites.

- **Saliva**

The mouth is kept moist and lubricated by saliva which flows to form a thin film (approximately 0.1mm deep) over all the internal surfaces of the oral cavity. Saliva plays the buffering role and Bicarbonate is the major buffering system in saliva. Phosphates, peptides and proteins are also involved. The mean pH of saliva is between pH 6.75 and 7.25, the pH and buffering capacity will vary with the flow rate. The slowest flow of saliva occurring during sleep.

The major organic constituents of saliva are **proteins and glycoproteins**, such as **mucin**, and they influence the oral microflora by:

- Adsorbing to the tooth surface to form a conditioning film (the acquired pellicle), when microorganisms are able to attach
- Acting as primary sources of nutrients (carbohydrates and proteins) for the resident microflora,
- Aggregating exogenous microorganisms, thereby facilitating their clearance from the mouth by swallowing,
- Inhibiting the growth of some exogenous microorganisms

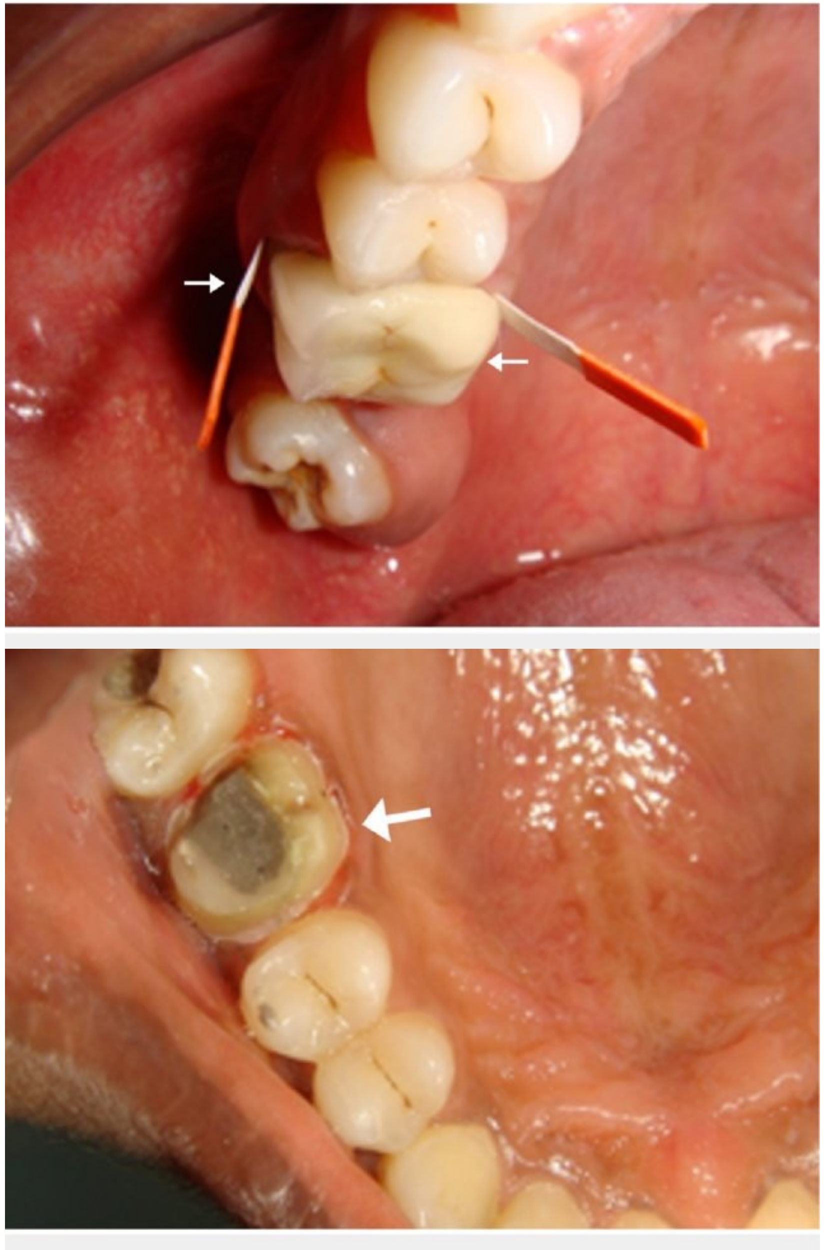
- Other **nitrogenous** compounds provided by saliva include urea and numerous amino acids. The concentration of free carbohydrates is low in saliva, and most oral bacteria produce glycosidases to degrade the side-chains of host glycoproteins. The metabolism of amino acids, peptides, proteins and urea can lead to the net production of alkalines, which contributes the acids production that resulted from fermentable carbohydrates.

- **Antimicrobial factors**, including lysozyme, lactoferrin, and the sialo peroxidase system, are present in saliva and play a key role in controlling bacterial and fungal colonization of the mouth. Antibodies have been detected, with **secretory IgA (sIgA) being the predominant class of immunoglobulin.**
- **IgG and IgM are also present but in lower concentrations**. A range of peptides with antimicrobial activity, including histidine-rich polypeptides (histatins), cystatins and defensins are also present in saliva.

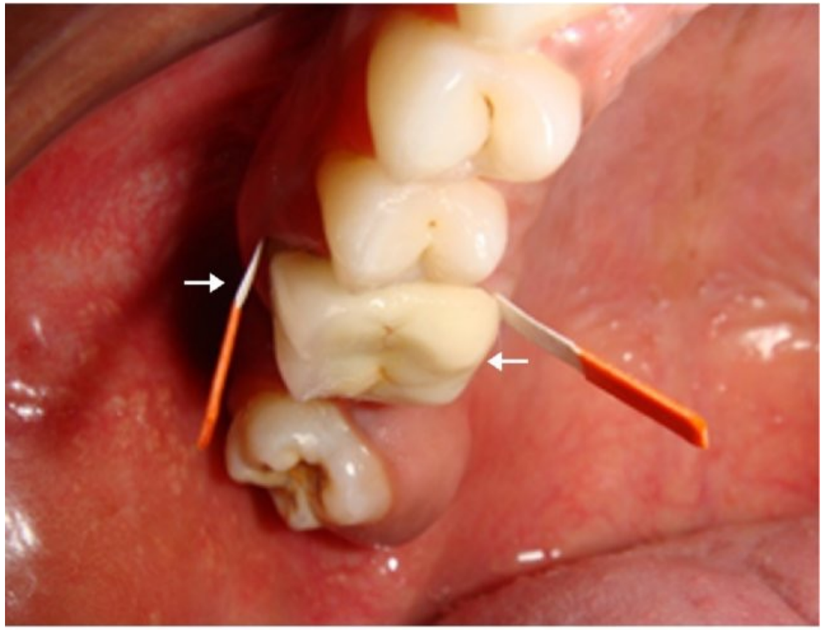
Gingival crevicular fluid (GCF)

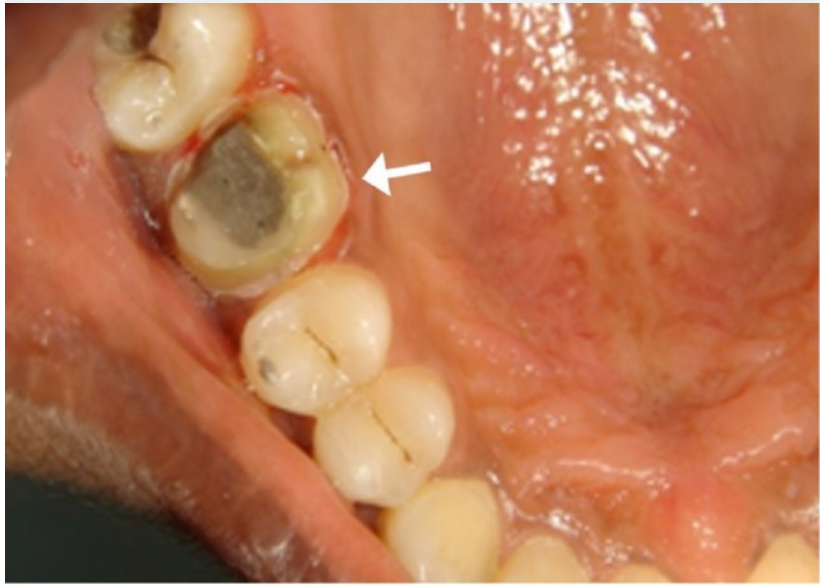
- Serum components can reach the mouth by the flow of a serum-like fluid through the junctional epithelium of the gingiva. The flow of **gingival crevicular fluid (GCF)** is relatively slow at healthy sites, increases in gingivitis and in advanced periodontal diseases, as part of the inflammatory response to the accumulation of plaque around the gingival margin.

- GCF can influence the microbial ecology of the site in a number of ways.* Its flow will remove non-adherent microbial cells, and *introduce components of the host defenses, especially IgG and neutrophils.



- GCF contains components of the host defenses. regulating the microflora of the gingival crevice in health and disease. **IgM and IgA are also present, as acomplement.** GCF contains leukocytes, of which 95% are neutrophils(can phagocytose bacteria within the crevice), the remainder being lymphocytes and monocytes





Factors Affecting Oral Microbial Growth

- ***Temperature***

The human mouth temperature is (35–36°C) provides stable conditions, suitable for the growth of microorganisms. Periodontal pockets with inflammation have a higher temperature (up to 39°C) compared with healthy sites. Small increasing in temperature can significantly alter bacterial gene expression, and possibly the competitiveness of individual species. A rise in temperature down-regulates the expression (gene downregulation is the process by **which a cell decreases the quantity of a gene expression product)** of some of the major proteases and fimbriae proteins in the periodontal pathogen, *Porphyromonas gingivalis*, and up-regulates (gene expression up-regulation is the process by which **a cell increases the quantity of a gene expression product)** synthesis of superoxide dismutase, which is involved in the neutralization of toxic oxygen metabolites.

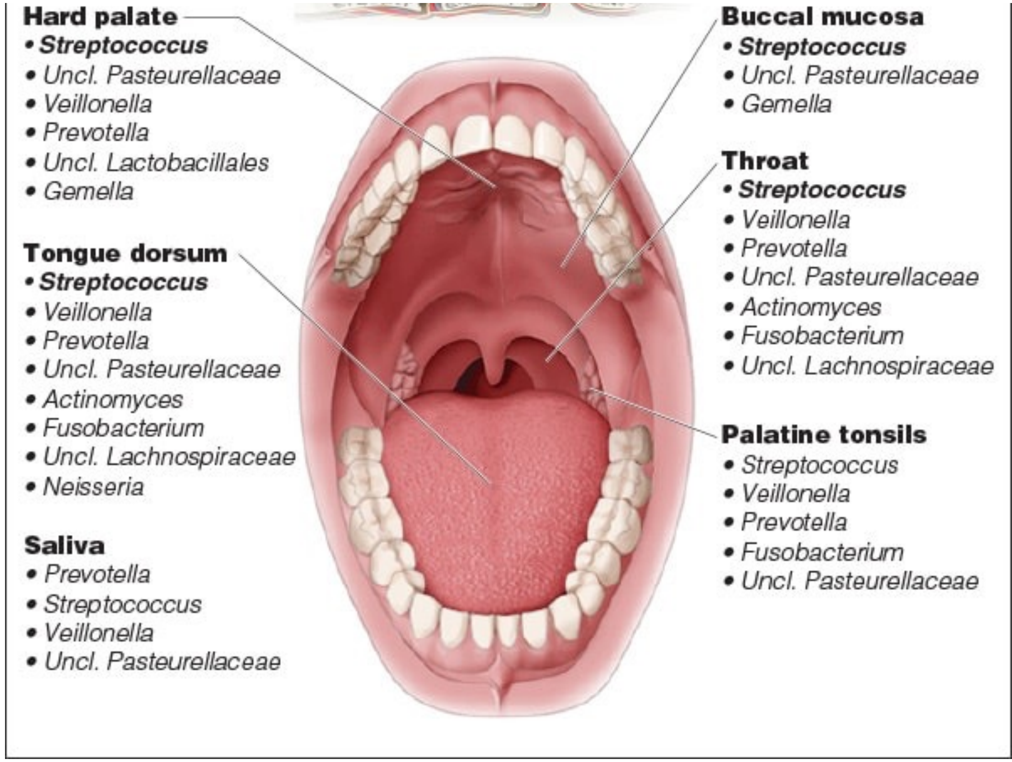
$$O_2^{\circ-} + 2H \xrightarrow{\text{dismutase}} O_2 + H_2O_2$$

- **Oxygen tension :**

Oxygen concentrations are very at different locations in the oral cavity.

As may be expected, the dorsum of the tongue and the buccal and palatal mucosa are in an essentially aerobic environment.

The oxygen tension inside a periodontal pocket is very low, with the species having a tendency to become reduced rather than oxidized, explaining the survival of obligate anaerobe. Therefore obligate aerobic organisms (which require oxygen) cannot survive, whereas obligate anaerobic organisms (which cannot tolerate the presence of oxygen) are able to thrive.



- **pH :**Shifts in the proportions of bacteria within dental plaque can occur following fluctuations in environmental pH .
- After sugar consumption, the pH in plaque can fall rapidly to below pH 5.0 by the production of lactic acid, the bacteria in plaque will be exposed to challenge of low pH .
- In many of the predominant plaque, bacteria that are associated with healthy sites can tolerate brief conditions of low pH, but are inhibited or killed by frequent or long exposures to acidic conditions. This can enhanced growth of, or colonization by, **acid-tolerant species, mutans streptococci and Lactobacillus** .
- In contrast, the pH of the gingival crevice can become alkaline during the host inflammatory response in periodontal disease, probably as a result of bacterial metabolism, e.g. ammonia production from urea. The pH of the healthy gingival crevice is 6.90, and rises to between pH 7.2 and 7.4 during disease. High PH can alter the pattern of gene expression in subgingival bacteria

- **Nutrients:**
- **(i) Endogenous nutrients** The persistence and diversity of the resident oral microflora is due primarily to the metabolism of the endogenous nutrients provided by the host, rather than by exogenous factors in the diet.

Saliva, which contains amino acids, peptides, proteins and glycoproteins vitamins and gases.

Gingival crevice is supplied with GCF(nutrients, such as albumin and other host proteins and glycoproteins, including haeme containing molecules) .

- **(ii) Exogenous** (dietary) nutrients ,fermentable carbohydrates are the only class of compound that markedly influence the ecology of the mouth. broken down to acids.

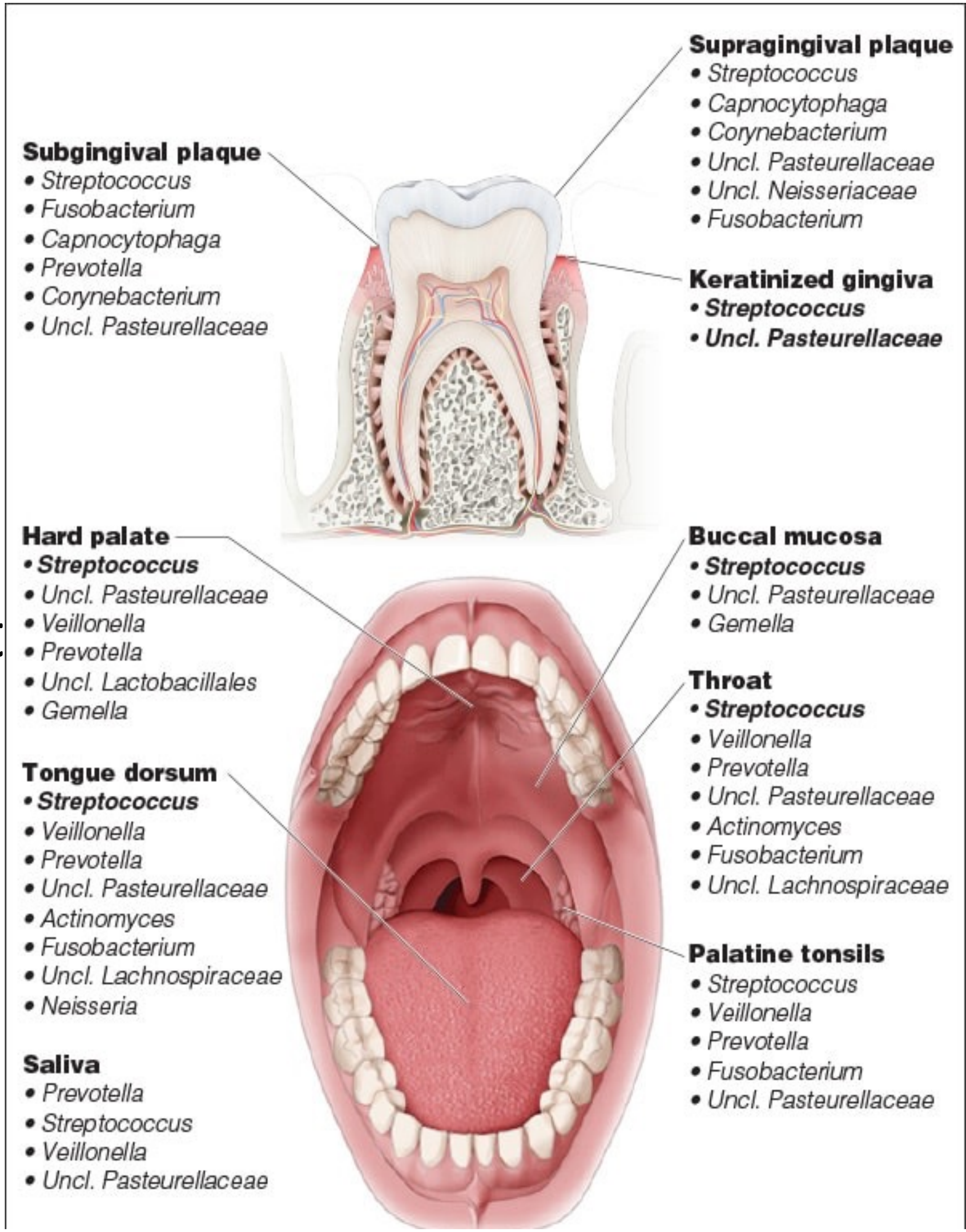
The levels of acid-tolerating species, especially mutans streptococci and lactobacilli, increase while the growth of acid-sensitive species (*Streptococcus sanguinis* and *S. gordonii*) is inhibited or decreased. This predispose a site to dental caries. Dairy products (milk, cheese) have some influence on the ecology of the mouth.

- **Host defenses**
- Mucosa (and enamel) a physical barrier to prevent penetration by microorganisms or antigens
- **(i) Innate immunity**: Chewing and the natural flow of saliva (or GCF in the gingival crevice) will remove microorganisms not firmly attached to an oral surface, and their physical removal by **swallowing** is an important defense mechanism., desquamation ensures that the bacterial load on most mucosal surfaces is light .

- **Mucins** agglutinate oral bacteria, interact with exogenous pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, as well as viruses including influenza virus.
- **Lysozyme** aggregate both Gram positive bacteria (including streptococci) and Gram negative periodontal pathogens. lyse bacteria by hydrolyzing peptidoglycan.
- **Chitinase** attacking yeast cell walls.
- **lactoferrin** bactericidal to a range of Gram positive and Gram negative bacteria and anti-inflammatory
- **Defensins** are a family of antibacterial peptides with a broad spectrum of antibacterial, antifungal and antiviral (including HIV) activity.
- **Cathelicidin** antimicrobial peptide that is secreted by epithelial cell.

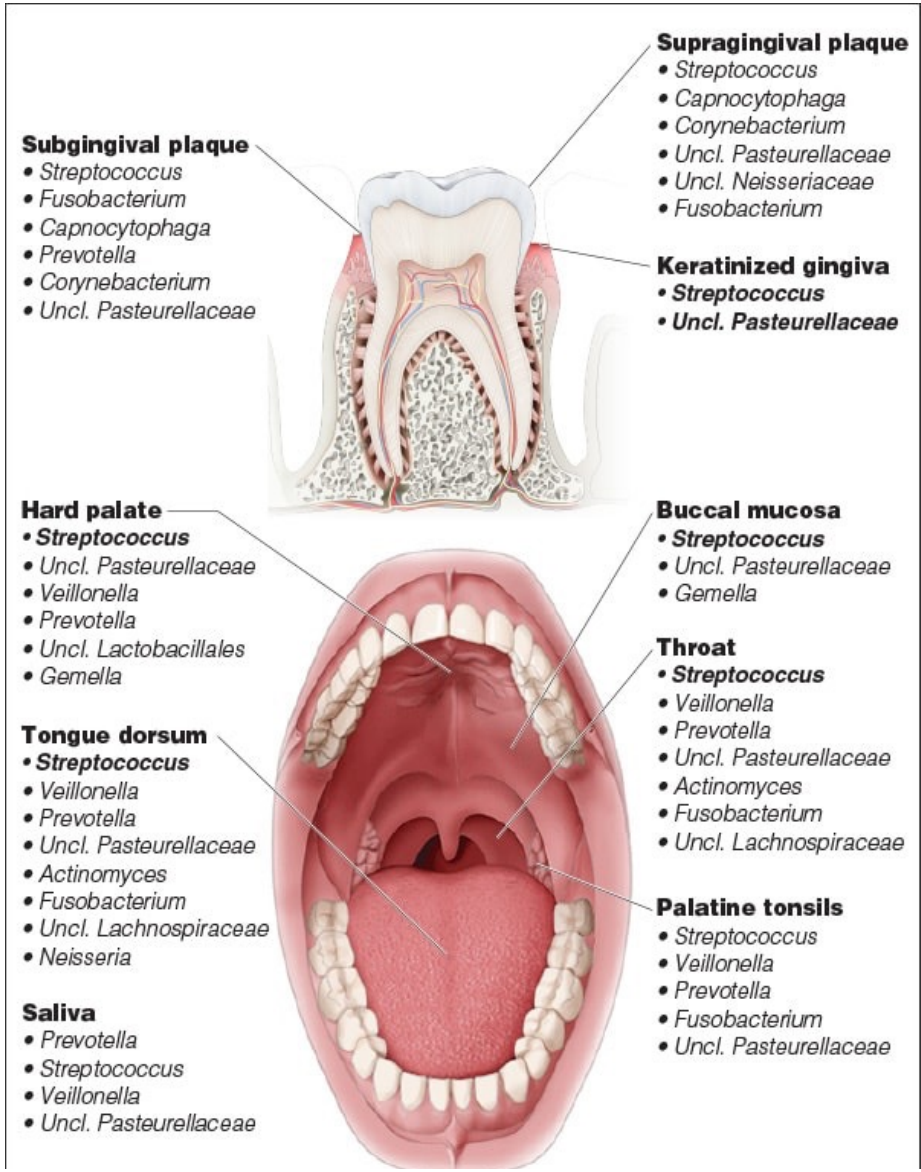
- **(ii) Adaptive immunity**: Components of the specific host defences (intraepithelial lymphocytes and Langerhans cells (tissue macrophage), immunoglobulins IgG and IgA) are found on and within the mucosa. where they act as a barrier to penetrating antigens. The predominant immunoglobulin in the healthy mouth is secretory IgA (sIgA), which is produced by plasma cells in the salivary gland. sIgA can agglutinate oral bacteria, modulate enzyme activity, and inhibit the adherence of bacteria to the buccal epithelium and to enamel. sIgA is usually considered to be a first line of defenses compared with other classes of immunoglobulin.

- **The resident oral microflora**
- **Gram positive** bacteria are commonly distributed on most surfaces of the mouth. The predominant genera are Streptococcus and Actinomyces; representative species are found at healthy sites, although many can also act as opportunistic pathogens. For example, mutans streptococci are implicated in dental caries .



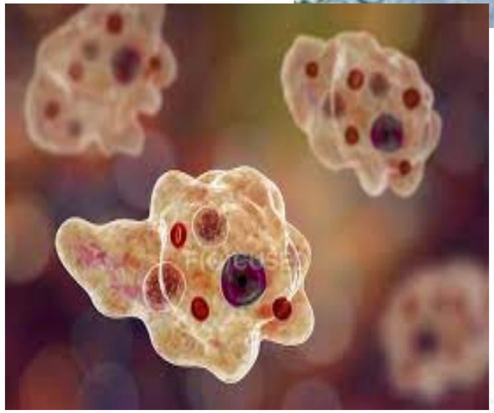
Other Gram positive cocci : *Enterococcus faecalis*

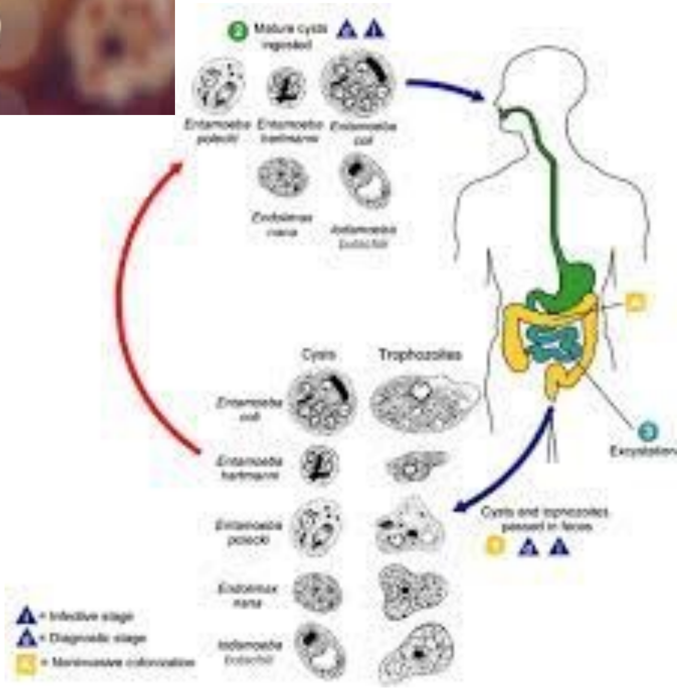
- **Oral Gram negative** bacteria are diverse, and include species that are facultatively and obligately anaerobic, as well as species that are microaerophilic and capnophilic. **Veillonella** are anaerobic Gram negative cocci that play an important role in dental plaque by converting lactate to weaker acids. Most of the anaerobic Gram negative bacilli are found in dental plaque, and have an a saccharolytic metabolism, and depend on proteins and glycoproteins for their nutrition; some common genera include Prevotella and Fusobacterium.



- **GRAM NEGATIVE COCCI:** Neisseria**,**Veillonella

- **Facultatively anaerobic and capnophilic genera**
- *H. parainfluenzae*
- **Obligately anaerobic genera :**Prevotella
- **FUNGI Aspergillus, Candida(** ***Candida albicans*** **)**
- **VIRUSES: Cytomegalovirus**
- **PROTOZOA:** ***Trichomonas tenax, Entamoeba gingivalis***





Oral flora changes with age

Time during a lifetime	MAJOR COMPONENTS & CHANGES IN ORAL FLORA
Newborn	Oral cavity sterile. Soon colonised by facultative and aerobic organisms; esp *S. salivarius*
6 months	Flora becomes more complex & includes anaerobic orgs eg. *Veillonella sp. & Fusobacteria*
Tooth eruption	Increase in complexity. *S sanguis, S mutans* and *A viscosus* appear. New habitats include hard surfaces and gingival crevice.
Child to adult	Various anaerobes frequently found inc. Members of the *Bacteroidaceae. Spirochaetes* isolated more frequently
Loss of teeth	Disappearance of *S mutan, S sanguis, spirochaetes* and many anaerobes
Dentures etc	Reappearance of bacteria able to grow on hard surfaces